



Determination of suspected fragrance allergens extended list by two-dimensional gas chromatography–mass spectrometry in ready-to-inject samples



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ABSTRACT

A new strategy was developed to elucidate and quantify 56 (69 analytes including isomers) suspected chemically defined fragrance allergens in perfumes that were recently targeted by the Scientific Committee on Consumer Safety (SCCS). Samples were analyzed with a two-dimensional gas chromatography–quadrupole mass spectrometry system (GC–GC–MS). Method performance was evaluated by the accuracy profile approach to determine uncertainties around the regulation limit of 10 mg/kg. This strategy was finally applied to 62 commercialized perfumes, analyzed in the routine workflow. Depending on the matrix, an acceptable result was obtained for 88–100% of the target analytes, which means that results were accurately defined under or above 10 mg/kg. This method saves considerable time for complete analysis and could be adopted for routine analysis due to its ruggedness and cost effectiveness.

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1. Introduction

The risk management of fragrance allergens is an important issue for the regulators and the cosmetic industry in Europe. The European regulation (EC) no. 1223/2009 on cosmetic products currently lists 26 potential fragrance allergens (24 chemically defined compounds) [1] for which information should be provided to consumers about their presence in cosmetic products, above 100 mg/kg in rinse-off products or 10 mg/kg in leave-on products.

The analytical method to quantify these compounds is based on gas chromatography–mass spectrometry (GC–MS) using two analytical columns of different polarity to limit the number of coelutions [2]. Because of this dual analysis and the complexity of fragrance matrices, data analysis is time consuming and requires trained users to accurately quantify coeluting compounds at the 10 mg/kg level. Recently, the Scientific Committee on Consumer Safety (SCCS) has issued an opinion which extends the number of substances that the consumer should be made aware of when they are present in cosmetic products. The list coming from this

opinion talks about 84 substances, including 28 natural extracts and 56 chemically defined compounds [3].

From an analytical perspective, this represents a huge challenge, creating a need for both a specific analytical method and more efficient data treatment. To our knowledge, there is no method for the analysis of this extended list in perfumes currently in the literature. For the cosmetics list, previous studies have described methods to improve analytical specificity, favoring multi-dimensional gas chromatography due to the complexity of cosmetic matrices. High-resolution mass spectrometry (HRMS) is another alternative, but the cost of these instruments can be prohibitive.

Two multi-dimensional approaches have been described for cosmetic analysis, comprehensive gas chromatography (GC × GC) [4–8] and heart-cut gas chromatography (GC–GC) [9–11], both coupled to mass spectrometry (MS). These approaches have the advantage of improved separation capacity compared to classic GC–MS methods [2,5,12–15]. GC × GC is the most powerful system to improve separation capacity, providing a complete 2D sample analysis, but it can be difficult to set up and requires very specific data processing, which is not the case with GC–GC.

Heart-cut methods have traditionally been used to improve chromatographic separation in small numbers of complex elution windows [9,10], but a multiple heart-cut strategy (14 cuts) was developed to detect 24 allergens in fragrances, in which every

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target compound was analyzed in 2D [11]. However, this work did not present quantitative results, and the system required two GC ovens, which is relatively bulky.

The multiple heart-cut strategy was expanded in this work to quantify 56 suspected allergens from the SCCS extended list in complex fragrances. The innovative aspect of this research is the combination of multiple heart-cut chromatography with a low thermal Mass chromatography module (LTM) applied to quantification of extended list of allergens. The LTM technology produces independent and fast temperature gradients in the second dimension using space-saving equipment. With different isomers selected, 69 analytes were monitored, and the quantitative results were evaluated with the accuracy profile methodology [16–18]. This statistical approach has already been successfully applied in various areas [19–22]. It estimates the total error of an analytical measurement and compares it to an acceptance limit. Moreover, the graphical representation obtained at the end of the validation is a useful decision-making tool for the analyst.

2. Experimental

2.1. Reagents, standards and samples

The target solutes (Table 1) were supplied by Sigma–Aldrich (St-Quentin Fallavier, France), BLH-PIM (St. Vallier De Thiey, France), Charabot (Grasse, France), DRT (Dax, France), Firmenich (Geneva, Switzerland), Givaudan (Vernier, Switzerland), IES (Allauch, France), IFF (New York, USA), Indukern (Barcelona, Spain), Mane (Le bar sur loup, France), Moellhausen (Vimerate, Italy), Niel (Grasse, France), PCW (Mouans-Sartoux, France), Robertet (Grasse, France), SIPA (Grasse, France), Symrise (Holzminden, Germany), Synarome (Chartres, France), Takasago (Ota, Japan) and Ventos (Barcelona, Spain). The total set of analytes consisted of 59 fragrance materials to be weighted in standard solutions. It included the 24 allergens indicated in EC regulation 1223/2009 and the other suspected allergens recently listed by the SCCS. We decided to add two other compounds, isodamascone because it has a similar chemical structure than other damascones listed by the SCCS and 6-methylcoumarin which is a material that should be prohibited according to IFRA (international fragrance association). Some regulated compounds consisted of more than one isomer monitored (e.g., citral = neral + geranial; farnesol = three isomers; tetramethyl acetyloctahydronaphthalene (iso E) = three isomers; beta damascone = two isomers; ebanol = two isomers; hexamethylindanopyran = two isomers; santalol = two isomers and terpineol = two isomers). Finally, 69 analytes were monitored in the method.

Preparation of standards and ISTDs solutions was based on the standardized method of the 24 allergens [2]. Allergen standards were mixed in two different stock solutions at approximately 5 g/L in 2-fluorotoluene (Merck, Darmstadt, Germany) to limit acetal formation: one containing the carbonyl compounds and the other containing non-carbonyl compounds. An internal standard solution of 1,4-dibromobenzene (VWR, Fontenay-sous-Bois, France) and 4,4'-dibromobiphenyl (Merck, Darmstadt, Germany) was prepared at 10 g/L in the same solvent. 2-Fluorotoluene was also used for intermediate dilution mixing both standard solutions of allergens (500 mg/L) and the final solution of ISTDs (100 mg/L). Then, a final dilution of standard allergens (50 mg/L) was made by dilution in ethanol (96.2% purity) supplied by Dislaub (Buchères, France) before preparing the calibration solutions (1–40 mg/L). Calibration solutions were made by an addition of different volumes of the final standard allergens solution and a fixed volume of the final ISTDs solution (100 µL) completed with ethanol to 1 mL. For the

perfumes, a sampling of 900 µL, weighted, was done with a 100 µL ISTDs final solution addition.

For validation, an “allergen-free” perfume was prepared (matrix A) with non-allergen raw materials frequently used in commercial perfumes (Supplemental Table S1), diluted in ethanol at 10% (w/w). The GC analysis of this homemade matrix was representative of a common fragrance with approximately 300 chromatographic peaks detected (ingredients + isomers + impurities). A commercialized perfume was also used as a second validation matrix (matrix B).

2.2. Instrumentation and analytical conditions

The GC–GC–FID–MS system was composed of an Agilent GC 7890A gas chromatograph equipped with an Agilent 7693 autosampler, a split/splitless injector, an Agilent LTM II series, a microfluidic heart-cut system (Deans switch) from Agilent, and an FID detector. It was coupled to an Agilent inert MSD 5975C with a triple axis detector (Palo Alto, USA). The first dimension separation was carried out on a VF1-MS capillary column (40 m × 0.15 mm i.d. × 0.150 µm film thickness, Agilent). The second dimension separation was performed on an LTM DB17-MS capillary column (20 m × 0.18 mm i.d. × 0.180 µm film thickness, Agilent). This system is illustrated in Fig. 1.

A strategy based on two injections of the same sample with two specific methods (M1 and M2) was developed. In such configuration, analytes were split between the two methods, conditions are described below. All parameters relative to the first dimension separation were the same for both methods: 1 µL split injection (15:1 ratio) at 250 °C with helium as the carrier gas at a 73 psi constant inlet head pressure. The GC oven temperature was programmed from 100 °C to 240 °C at 10 °C/min and a final ramp to 300 °C at 25 °C/min (held 34 min). In both methods, the first GC column was connected to the Deans switch, where a constant helium pressure of 45 psi was applied to guide samples either to the FID (260 °C; H₂ flow: 45 mL/min; air flow: 450 mL/min) or to the second dimension through specific cut programs (Fig. 2).

Two specific 2D temperature programs were optimized for each method: M1 was set from 30 °C (held 7.5 min) to 200 °C (held 0.2 min) at 300 °C/min, to 30 °C (held 16.17 min) at 300 °C/min, to 100 °C at 65 °C/min, to 180 °C at 5 °C/min, and a final ramp to 280 °C (held 5 min) at 30 °C/min. M2 method was programmed from 30 °C (held 25 min) to 100 °C at 65 °C/min, to 180 °C at 5 °C/min, and a final ramp to 280 °C (held 5 min) at 30 °C/min.

The transfer line temperature to the mass spectrometer was set at 300 °C, ion source was heated at 230 °C and the MS was operated in SIM/scan mode, scanning between 40 and 300 amu. Selected ions for the SIM programs are shown in Table 1.

2.3. Validation protocol

Quantitative results were evaluated using the accuracy profile method [16–18] (see Supplementary data, Fig. S1, for more). It is a useful tool that aids the analyst in determining whether method performance is compliant with requirements. It is based on an evaluation of the trueness and intermediate precision. The acceptance limit (red lines in Supplemental Fig. S1) is a threshold value, fixed by users in relation to the method objectives, and the tolerance limit (green lines in Supplemental Fig. S1) is an interval assumed to contain a known part of the results that will be generated by the method [16–18]. This interval is called β and was fixed at 90% in our study. It was admitted that resulting tolerance limits should be within the acceptance limit set at $\pm 35\%$.

Five calibration levels (1, 5, 10, 20 and 40 mg/L), four levels of spiked matrix (5, 10, 15 and 30 mg/L) and a non-spiked level were injected in triplicate. Operation was repeated during three

Table 1
Compound list, monitored ions and detection method.

Key	Compound	CAS number	SIM Ions			Detection method	ISTD quantification	Key	Compound	CAS number	SIM Ions			Detection method	ISTD quantification		
1	Alpha pinene	80-56-8	93 ^a	121	136	M1	ISTD1	38	Coumarin	91-64-5	118 ^a	146	90	M2	ISTD2		
2	Benzaldehyde	100-52-7	106 ^a	105	77	M1	ISTD1	39	Isoeugenol	97-54-1	164 ^a	149	131	M2	ISTD2		
3	Beta pinene	127-91-3	93 ^a	121	136	M1	ISTD1	40	Ebanol 1 ^b	67801-20-1	121 ^a	149	164	M1	ISTD1		
4	Benzyl alcohol	100-51-6	79 ^a	108	107	M2	ISTD1	41	Beta caryophyllene	87-44-5	93 ^a	133	189	M1	ISTD1		
5	Alpha terpinene	99-86-5	136 ^a	121	93	M2	ISTD1	42	Ebanol 2 ^b	67801-20-1	121 ^a	107	93	M1	ISTD1		
6	Limonene	138-86-3	68 ^a	93	136	M2	ISTD1	43	Alpha isomethyl ionone	127-51-5	206 ^a	191	107	M2	ISTD1		
7	Salicylaldehyde	90-02-8	121 ^a	122	93	M2	ISTD1	44	Eugenyl acetate	93-28-7	164 ^a	149	131	M2	ISTD2		
8	Linalool	78-70-6	71 ^a	93	121	M2	ISTD1	45	Butylphenyl methylpropional	80-54-6	189 ^a	147	204	M1	ISTD2		
9	Terpinolene	586-62-9	136 ^a	121	93	M2	ISTD1	46	6-Methyl coumarin	92-48-8	160 ^a	132	104	M2	ISTD2		
10	Camphor	76-22-2	108 ^a	95	152	M2	ISTD1	47	Amyl salicylate	2050-08-0	120 ^a	138	208	M2	ISTD2		
11	Methyl 2-octynoate	111-12-6	95 ^a	66	139	M2	ISTD1	48	Propylidene phtalide	17369-59-4	159 ^a	174	104	M2	ISTD2		
12	Menthol	1490-04-6	138 ^a	95	123	M2	ISTD1	49	Isoeugenyl acetate	93-29-8	164 ^a	149	206	M1	ISTD2		
13	Alpha terpineol	8000-41-7	93 ^a	121	136	M1	ISTD1	50	Amyl cinnamal	122-40-7	129 ^a	202	173	M2	ISTD2		
14	Methyl salicylate	119-36-8	120 ^a	92	152	M1	ISTD1	51	Hydroxyisohexyl-3-cyclohexene carboxaldehyde (HICC)	31906-04-4	136 ^a	192	177	M2	ISTD2		
15	Gamma terpineol	8000-41-7	136 ^a	121	93	M1	ISTD1	52	Amyl cinnamyl alcohol	101-85-9	204 ^a	129	117	M1	ISTD2		
16	Citronellol	106-22-9	69 ^a	123	138	M2	ISTD1	53	Farnesol 2 (cis) ^b	4602-84-0	69 ^a	93	136	189	M1	ISTD2	
17	Neral (citral)	5392-40-5	69 ^a	94	109	M2	ISTD1	54	Tetramethyl acetyloctahydronaphtalene 1 ^b	54464-57-2	191 ^a	119	234	M1	ISTD2		
18	Linalyl acetate	115-95-7	93 ^a	121	136	M2	ISTD1	55	Farnesol 3 ^b	4602-84-0	69 ^a	93	136	189	M1	ISTD2	
19	Carvone	99-49-0	150 ^a	82	108	M1	ISTD1	56	Alpha santalol	115-71-9	93 ^a	121	107	189	M1	ISTD2	
20	Geraniol	106-24-1	69 ^a	93	123	M1	ISTD1	57	Tetramethyl acetyloctahydronaphtalene 2 ^b	54464-57-2	191 ^a	121	234	M2	ISTD2		
21	Geranial (citral)	5392-40-5	69 ^a	84	94	M2	ISTD1	58	Farnesol 1 (trans) ^b	4602-84-0	136 ^a	69	93	189	M2	ISTD2	
22	Cinnamal	104-55-2	131 ^a	103	77	M2	ISTD1	59	Tetramethyl acetyloctahydronaphtalene 3 ^b	54464-57-2	135 ^a	191	234	M1	ISTD2		
23	Hydroxycitronellal	107-75-5	139 ^a	121	71	59	M2	ISTD1	60	Beta santalol	77-42-9	94 ^a	79	122	189	M1	ISTD2
24	Anise alcohol	105-13-5	138 ^a	109	121	M2	ISTD1	61	Hexyl cinnamal	101-86-0	216 ^a	129	145	M2	ISTD2		
25	Anethol	4180-23-8	148 ^a	117	133	M1	ISTD1	62	Benzyl benzoate	120-51-4	212 ^a	194	105	91	M1	ISTD2	
26	Cinnamyl alcohol	104-54-1	92 ^a	134	115	M1	ISTD1	63	Acetylcedrene	32388-55-9	161 ^a	231	246	M2	ISTD2		
27	Dimethylbenzyl carbiny acetate (DMBCA)	151-05-3	132 ^a	91	117	M2	ISTD1	64	Hexamethylindanopyran 1 ^b	1222-05-5	243 ^a	213	258	M1	ISTD2		
28	Eugenol	97-53-0	103 ^a	131	137	M1	ISTD1	65	Hexamethylindanopyran 2 ^b	1222-05-5	243 ^a	213	258	M1	ISTD2		
29	Geranyl acetate	105-87-3	69 ^a	93	136	M1	ISTD1	66	Benzyl salicylate	118-58-1	91 ^a	65	228	M1	ISTD2		
30	Delta damascene	57378-68-4	69 ^a	123	192	M2	ISTD1	67	Hexadecanolactone	109-29-5	55 ^a	83	97	M2	ISTD2		
31	Damascenone	23696-85-7	190 ^a	121	105	M2	ISTD1	68	Benzyl cinnamate	103-41-3	131 ^a	192	238	M1	ISTD2		
32	Vanillin	121-33-5	151 ^a	109	123	M2	ISTD2	69	Sclareol	515-03-7	109 ^a	177	191	290	M2	ISTD2	
33	Isodamascone	39872-57-6	192 ^a	123	69	M2	ISTD1	ISTD1	1,4-Dibromobenzene	106-37-6	236 ^a	155	75	M1+M2			
34	Alpha damascene	43052-87-5	192 ^a	81	123	M2	ISTD1	ISTD2	4,4'-Dibromobiphenyl	92-86-4	152 ^a	76	151	M1+M2			
35	Beta 2 damascene ^b	23726-92-3	192 ^a	177	123	M2	ISTD1										
36	Majantol	103694-68-4	106 ^a	91	178	M1	ISTD1										
37	Beta 1 damascene ^b	23726-92-3	177 ^a	123	192	M1	ISTD1										

^a Preferential ion for quantification, if not coeluted.

^b Isomers grouped into classes based on their relative abundance in the standard.

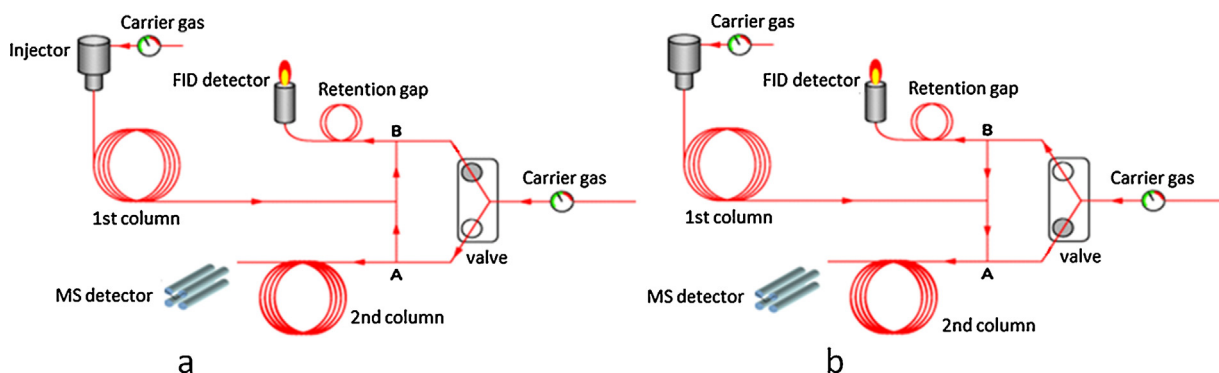


Fig. 1. Instrumental diagram for the GC–GC–FID–MS. In (a), the valve is closed, and compounds are eluted through the 1st column to the FID. In (b), the valve is open, and compounds are eluted through the 1st column to the 2nd column, and then to the MS.

series. This experimental design was successively applied to both the allergen-free and commercialized perfumes. To our purpose, which was to determine if fragrance allergens were present under or above the regulated concentration of 10 mg/kg, the accuracy was needed around this concentration, and the validation plan was built accordingly.

2.4. Data processing

Quantitation was performed using Agilent MSD ChemStation GC–MS Data Analysis software combined with QuantBrowser (Leoson BV, Netherland). QuantBrowser software is a useful add-on for processing data in batches. This visualization saves time and secures quantitation results with color-coded warnings for incorrect ion ratios.

3. Results and discussion

3.1. Chromatographic separation strategy and MS detection

Reduced i.d. capillary columns were used (1D: 40 m × 0.15 mm i.d. and 2D: 20 m × 0.18 mm i.d.) to achieve both high resolution and rapid analysis. The stationary phases of the columns were selected based on a compromise between orthogonality and robustness of the method.

The developed strategy allowed to quantify fragrance allergens in perfumes in compliance with the regulatory limit of 10 mg/kg. All parameters were optimized for this level of performance. The complexity of this analysis is illustrated in Fig. 3(a), which presents a 1D chromatogram with FID detection of a standard mixture at a

concentration near the regulation limit compared to a chromatogram of matrix A. Chromatograms in Fig. 3(b and c) illustrate more specific results obtained thanks to the two methods strategy, 2D and SIM mode.

To develop a method that was simple and robust for a daily use, no cryofocalization device was installed. Focalization was not necessary except with the three most volatile compounds (no. 1–3). These molecules were not retained well enough by the column and started eluting through the second column at 30 °C. A rapid separation in the first method overcame this problem. The LTM module could heat and cool with temperature ramps of 300 °C/min, effectively separating these volatile compounds and returning to initial temperatures in less than 1.5 min before the next target allergens eluted from the first column.

Another challenge was the wide concentration ranges (trace to several percent) of both target analytes and interferences that could be present in samples. Especially compound at high concentration resulted in distorted and wide peaks, leading to potential coelutions. To increase specificity against potential interferences, SIM programs were built by selecting ions specific to the target analytes and excluding interfering ions, determined from the study of different matrices.

Quantification was then based on target and qualifier ion ratios: if they did not correlate with expectations [23], the compound identification was performed manually in scan mode to determine the deviation, which may include coelutions, concentrations outside of the calibration range or detector saturations. For identified coelutions, quantification was based on the most specific ion monitored if possible or classed as undetermined if not.

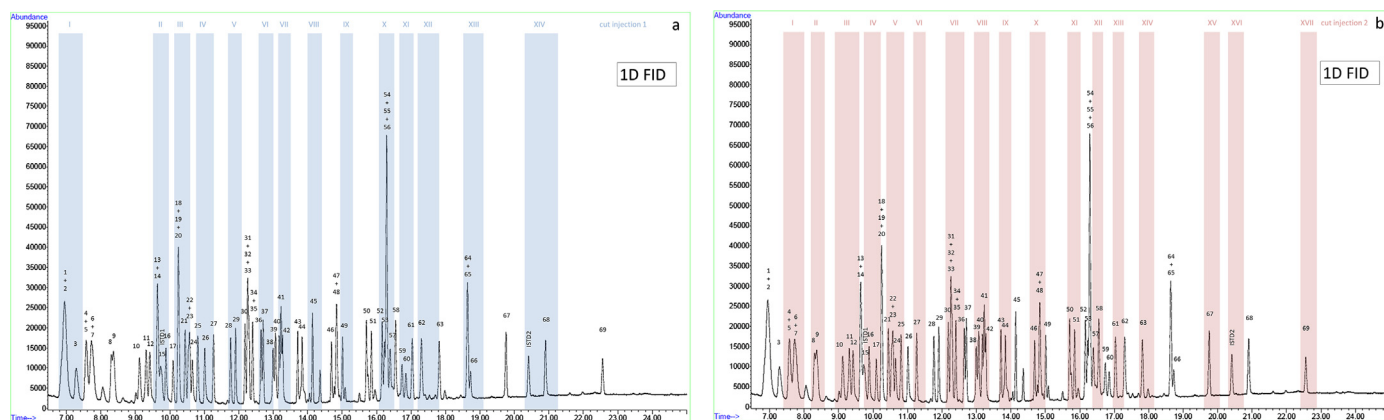


Fig. 2. 1D GC–FID chromatograms of a standard mixture of the fragrance allergens at 10 mg/L in ethanol (see number code equivalence in Table 1). The cut segments are illustrated for each method M1 (a) and M2 (b).

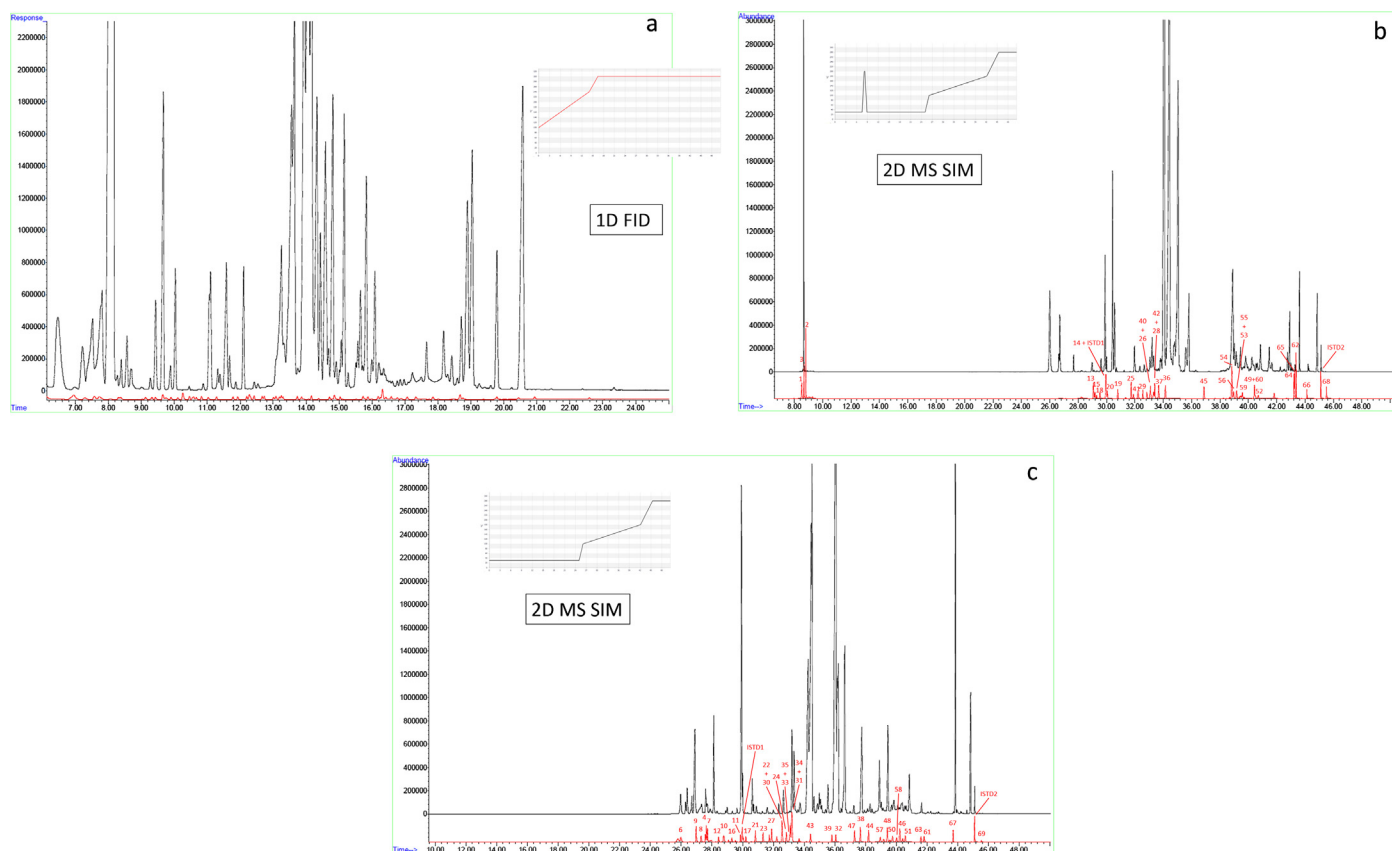


Fig. 3. Overlain chromatograms of a 10 mg/L mixture of standard allergens (red) and matrix A (black) in 1D FID without cuts (a) and 2D MS SIM for M1 (b) and M2 (c). Respective temperature programs are embedded within the chromatograms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This GC–GC and MS optimization resulted in a quantitative method that can be considered to be globally accurate to analyze perfumes without sample preparation. It should be noted that analytes with poor ion specificity remained difficult to identify in some samples, particularly farnesol, santalol and HICC.

3.2. Validation of quantification results

3.2.1. Validation criteria

Some of the suspected fragrance allergens are very common raw materials used in perfumes that is why, to our knowledge, no commercial perfume free of allergens was available. However, to evaluate method performances and especially recoveries for all the analytes, there was a need to know precisely the quantity of allergens present in a representative solution. Matrix A was made in this goal and was systematically injected unspiked to determine traces of allergens that may be present due to impurities in raw materials used in its preparation. If traces were detected, they were subtracted from the results of spiked samples during data processing. Then following the accuracy profile protocol, for each analytes, means and uncertainties were calculated and graphically represented.

To confirm results or discard some unexpected behavior due to the matrix A, a second matrix (B), was studied. As the matrix B was a commercialized perfumes, some suspected fragrance allergens were identified in its composition. For analytes that were present in low quantity, an unspiked injection was used to subtract this quantity in spiked samples, as for matrix A. However, sixteen analytes were present at high concentrations above the spiked levels, and

data for those compounds could not be included in the validation method.

The complete validation represented a total of 396 analytical runs and 11,700 verified analytes. Results were considered to be in accordance with criteria when the tolerance limits were within the acceptance limits for, at least, a concentration range including 10 mg/L.

3.2.2. Matrix A results

Results of the validation protocol, based on matrix A (“allergen-free”), are illustrated in Fig. 4. Of the 69 target compounds, 40 had a quantification range that was strictly in accordance with the criteria. Eight more compounds had a quantification range or uncertainties near the criteria as their acceptance limits were within a tolerance limit of $\pm 45\%$ or less (amyl salicylate, ebanol 2, geranial, hexylcinnamal, isoeugenol, linalyle acetate, menthol and salicyladehyde).

For the 21 other analytes, different kind of results were obtained and can be categorized by behavior. First of all, no data were obtained for sclareol because it appeared to be completely degraded in this matrix, this was not observed in others matrices used in the study. Perhaps this reaction with the matrix could explain other non-compliant results, some analytes which contains hydroxyl groups were also clearly underestimated (anise alcohol, menthol, beta santalol, cinnamyl alcohol, geraniol and citronellol).

Another group of non-compliant results can be made with amyl cinnamal, butylphenyl methylpropional, coumarin, delta damascene, eugenol, isoeugenyl acetate, alpha isomethylionone and majantol. They were characterized by a profile with overestimated means and large uncertainties due to the increase of their responses

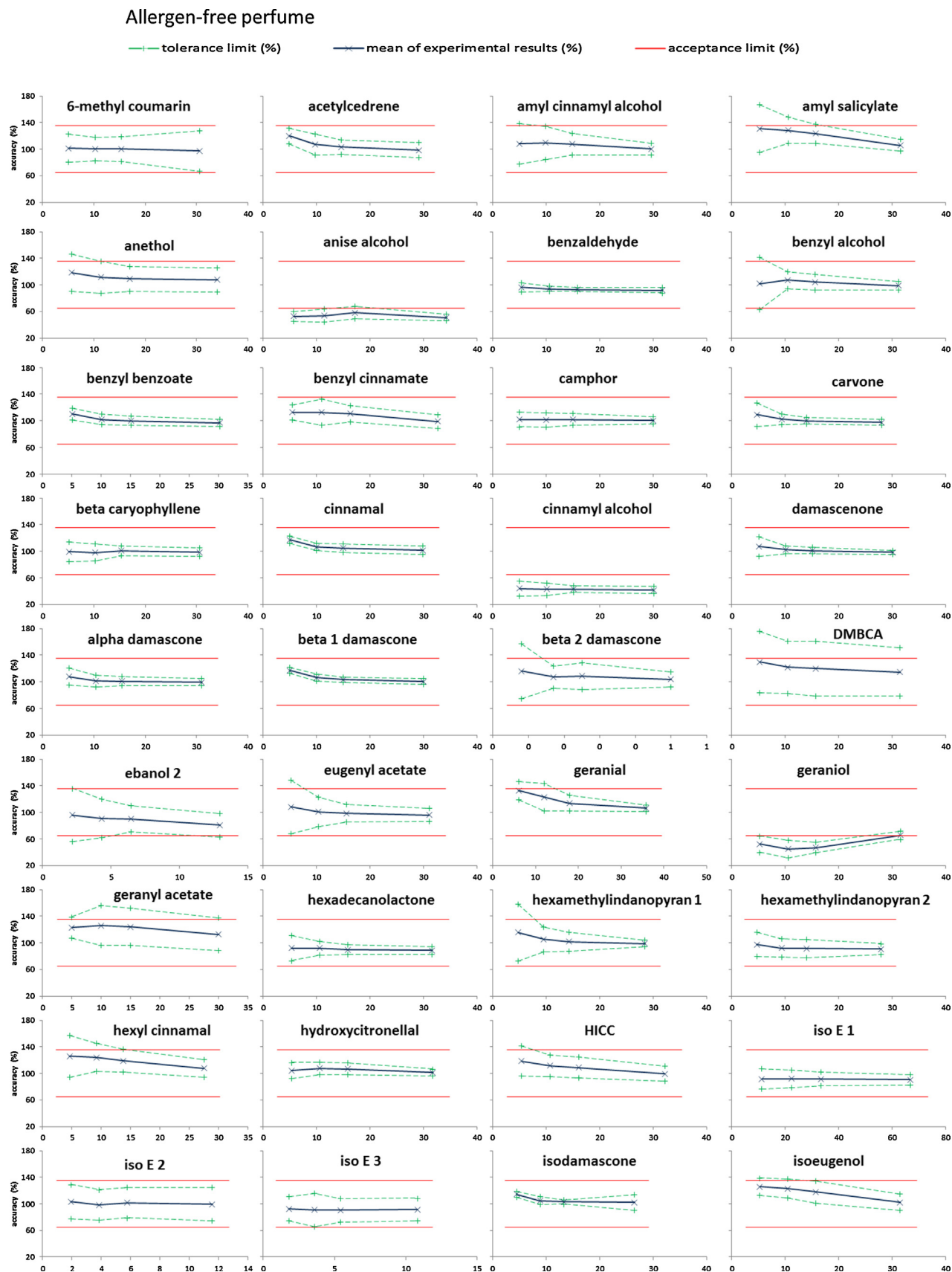


Fig. 4. Accuracy profiles of the 69 target analytes in matrix A.

Allergen-free perfume

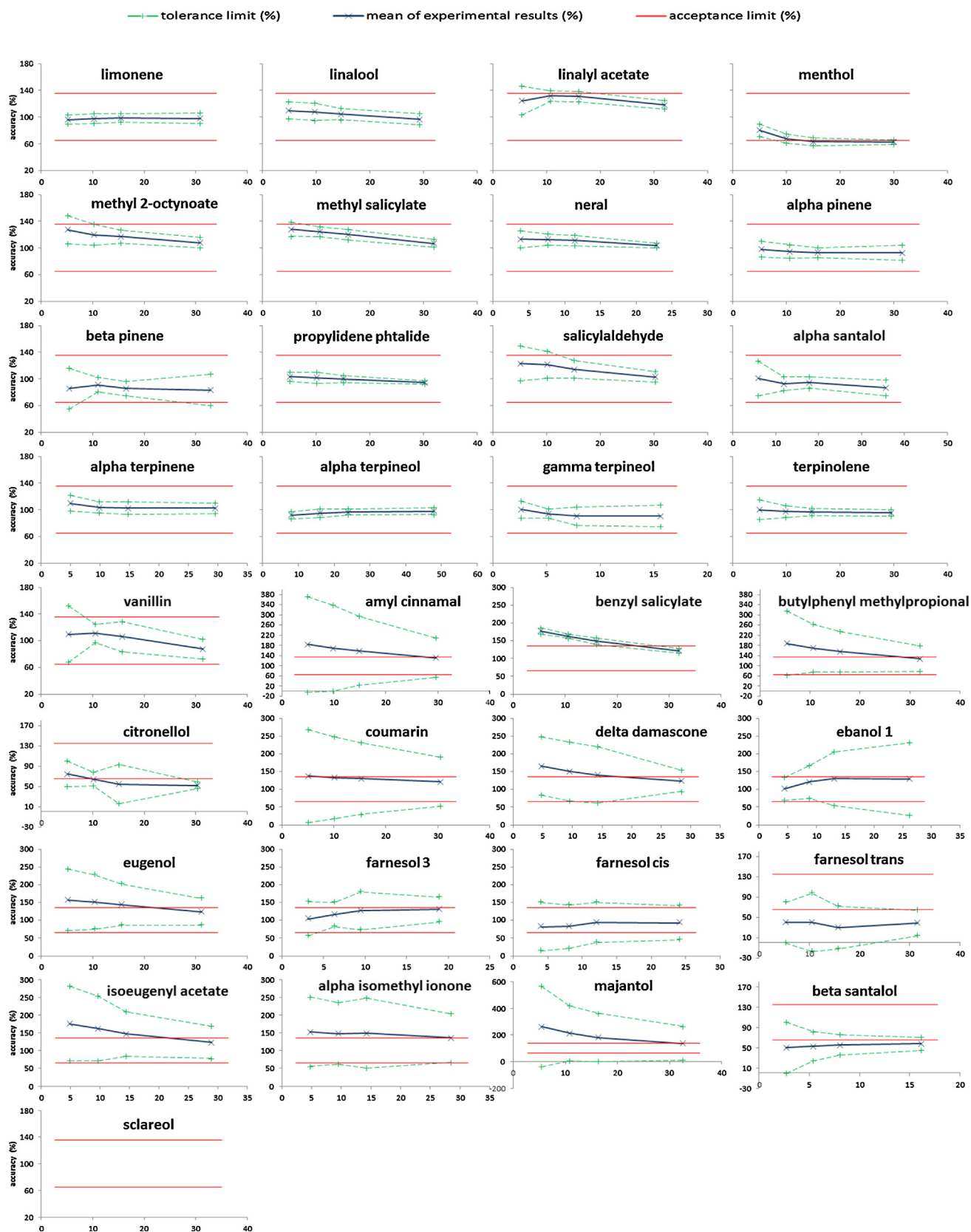


Fig. 4. (Continued)

Table 2
Summarized results of the 62 commercialized perfumes analysis.

Sample	Number of analytes < 10 mg/kg	Number of analytes > 10 mg/kg	Number of undetermined analytes ^a	Sample	Number of analytes < 10 mg/kg	Number of analytes > 10 mg/kg	Number of undetermined analytes ^a
PF1	43	22	4	PF32	39	27	3
PF2	36	28	5	PF33	42	27	0
PF3	32	32	5	PF34	35	35	1
PF4	32	34	3	PF35	42	27	0
PF5	29	35	5	PF36	52	17	0
PF6	37	27	5	PF37	51	18	0
PF7	42	22	5	PF38	38	28	3
PF8	36	27	6	PF39	45	24	0
PF9	38	25	6	PF40	38	28	3
PF10	37	26	6	PF41	37	28	4
PF11	35	26	8	PF42	30	33	6
PF12	34	30	5	PF43	33	32	4
PF13	35	28	6	PF44	29	34	6
PF14	32	32	5	PF45	34	32	3
PF15	39	27	3	PF46	37	32	0
PF16	33	33	3	PF47	39	30	0
PF17	27	39	3	PF48	35	31	3
PF18	38	26	5	PF49	42	23	4
PF19	44	19	6	PF50	41	24	4
PF20	36	28	5	PF51	44	25	0
PF21	35	29	5	PF52	46	17	6
PF22	34	30	5	PF53	40	24	5
PF23	29	35	5	PF54	36	28	5
PF24	27	38	4	PF55	32	32	5
PF25	24	38	7	PF56	45	19	5
PF26	41	23	5	PF57	32	31	6
PF27	40	25	4	PF58	28	33	8
PF28	33	28	8	PF59	42	21	6
PF29	41	23	5	PF60	30	33	6
PF30	44	24	1	PF61	37	27	5
PF31	41	24	4	PF62	35	30	4

^a Classified as undetermined when uncertainties on detection, identification or quantification was too important (mainly because of coelutions).

during the different days of the validation protocol. The mechanism behind this is not known but seems to be related to interactions within the matrix once again.

Finally benzyl salicylate, ebanol 1 and farnesols had non-compliant results, which are explained by coelutions.

3.2.3. Matrix B results

Accuracy profiles for the matrix B (commercialized perfume) are available in the Supplementary data (Supplemental Fig. S2). In this matrix, 16 analytes were too concentrated to be taking into account regarding spiked amounts (benzyl benzoate, benzyl salicylate, beta caryophyllene, cinnamyl alcohol, citronellol, coumarin, eugenol, geraniol, geranyl acetate, hydroxycitronellal, alpha isomethyl ionone, limonene, linalool, linalyl acetate, beta pinene and vanillin).

For all the other analytes, the objective was to confirm that unexpected accuracy profiles observed in the matrix A were not reproduced in the matrix B. Means of results were all within the acceptance limit except for anise alcohol, ebanol 1 and sclareol which were overestimated.

In addition to these three compounds, amyl cinnamyl alcohol, eugenyl acetate, farnesol 3, isoeugenol, majantol, alpha and beta santalol presented uncertainties slightly over the acceptance limit. These compounds were already present in matrix B composition or were coeluted, explaining the more important uncertainties. The other 43 analytes gave compliant results.

Finally, five compounds could not be measured with low uncertainty in either matrix: anise alcohol, ebanol 1, isoeugenol, majantol and beta santalol. Nine others yielded non-compliant results in one matrix, but had no data in the other, resulting in inconclusive method performance for these compounds. To conclude on this method performances evaluation, 50 analytes gave acceptable results in at least one matrix, quantitation of ebanol and santalol

could be based on validated isomers as a priority and four compounds had uncertainties between $\pm 45\%$, which remains a workable result.

3.3. Perfume sample analyses

After validation, the strategy was routinely applied to 62 commercialized perfumes. For clarity and conciseness, results were summarized in the Table 2, detailed results could be presented in a forthcoming communication. The majority of the analytes could be determined under or above the 10 mg/kg regulated limit (88–100%). Depending on the matrix composition and complexity, some unexpected coelutions remain inevitable and some analytes cannot be clearly identify or quantify with a low uncertainty in these samples. To solve these difficult cases, complementary solutions are needed and specific sample preparation is investigated.

The total time for complete sample analysis was approximately 2 h, including direct sample injection, two analytical runs of 51 min each and approximately 10 min for data processing. This represents a significant time saving process.

4. Conclusion

A GC–GC–MS strategy using two methods without sample preparation was optimized and evaluated using accuracy profiles to determine 56 (69 analytes including isomers) suspected fragrance allergens in perfume samples.

The optimized strategy was successful in quantifying the target allergens in complex perfume matrices. Most of the target compounds complied with the validation criteria, illustrating the robustness of the method at the regulatory limit of 10 mg/kg. Due to the 2D chromatographic separation and strategy implementation, this method gave data fast to be processed for perfume samples.

Matrix effects, specifically for compounds present in large concentrations and coeluting with other target compounds, were observed and could be a limitation for compound identification.

These results, obtained with a quadrupole detector and without sample preparation or cryofocusing, illustrate a method that is both robust and cost-effective. However, some compounds can be problematic in certain matrices, and we are currently working on very specific sample preparation procedures that could be used in these special cases. Instruments with higher resolving power, such as high-resolution MS, could improve the specificity of the method, but these instruments are a significant investment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2015.05.045>

References

- [1] European Union, Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products, 2009.
- [2] European Standards (EN), EN 16274-2012 Methods for Analysis of Allergens. Quantification of Suspected Fragrance Allergens in Consumer Products. Step 1: GC Analysis of Ready-to-Inject Sample, 2012.
- [3] Scientific Committee Consumer Safety (SCCS), 1459/11 Opinion on Fragrance Allergens in Cosmetic Products – 15th Plenary Meeting, 2012.
- [4] C. Debonneville, A. Chaintreau, Quantitation of suspected allergens in fragrances: part II. Evaluation of comprehensive gas chromatography–conventional mass spectrometry, *J. Chromatogr. A* 1027 (2004) 109–115.
- [5] C. Debonneville, M. Thomé, A. Chaintreau, Hyphenation of quadrupole MS to GC and comprehensive two-dimensional GC for the analysis of suspected allergens: review and improvement, *J. Chromatogr. Sci.* 42 (2004) 450–455.
- [6] C. Cordero, C. Bicchi, D. Joulain, P. Rubiolo, Identification, quantitation and method validation for the analysis of suspected allergens in fragrances by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry and with flame ionization detection, *J. Chromatogr. A* 1150 (2007) 37–49.
- [7] L. Mondello, Analysis of perfume allergens by using comprehensive 2D GC and a rapid-scanning quadrupole mass spectrometer, *Shimadzu Appl. Note* (2009) 1–4.
- [8] L. Mondello, A. Casilli, P.Q. Tranchida, G. Dugo, P. Dugo, Comprehensive two-dimensional gas chromatography in combination with rapid scanning quadrupole mass spectrometry in perfume analysis, *J. Chromatogr. A* 1067 (2005) 235–243.
- [9] F. David, M. Klee, Suspected Flavor and Fragrance Allergens in Perfumes Using Two-Dimensional GC with Independent Column Temperature Control Using an LTM Oven, *Agil. Technol. Publ.* 5990-3576E, 2009.
- [10] C. Devos, N. Ochiai, K. Sasamoto, P. Sandra, F. David, Full evaporation dynamic headspace in combination with selectable one-dimensional/two-dimensional gas chromatography–mass spectrometry for the determination of suspected fragrance allergens in cosmetic products, *J. Chromatogr. A* 1255 (2012) 207–215.
- [11] L. Mondello, A. Casilli, P.Q. Tranchida, D. Sciarrone, P. Dugo, G. Dugo, Analysis of allergens in fragrances using multiple heart-cut multidimensional gas chromatography–mass spectrometry, *LCGC Eur.* 21 (2008).
- [12] S. Kanani, A. Magne, Détection et dosage de composés allergènes dans des compositions de parfumerie par couplage chromatographie en phase gazeuse–spectrométrie de masse, *Spectra Anal.* 248 (2006) 28–38.
- [13] W. Luan, C. Sandy, M. Szelewski, Determination of Allergens in Fragrance Products Using Agilent Deconvolution Reporting Software, *Agil. Technol. Publ.* 5989-8724E, 2008.
- [14] L. Sanchez-Prado, M. Llompart, J.P. Lamas, C. Garcia-Jares, M. Lores, Multi-component analytical methodology to control phthalates, synthetic musks, fragrance allergens and preservatives in perfumes, *Talanta* 85 (2011) 370–379.
- [15] M. Celeiro, E. Guerra, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Development of a multianalyte method based on micro-matrix–solid-phase dispersion for the analysis of fragrance allergens and preservatives in personal care products, *J. Chromatogr. A* 1344 (2014) 1–14.
- [16] M. Feinberg, Validation of analytical methods based on accuracy profiles, *J. Chromatogr. A* 1158 (2007) 174–183.
- [17] AFNOR, NF V03-110, Analyse des produits agricoles et alimentaires. Protocole de caractérisation en vue de la validation d'une méthode d'analyse quantitative par construction du profil d'exactitude (2010).
- [18] M. Feinberg, Labo-stat: guide de validation des méthodes d'analyse, Tec & Doc Lavoisier, Paris, 2009.
- [19] R.D. Marini, A.-C. Servais, E. Rozet, P. Chiap, B. Boulanger, S. Rudaz, J. Crommen, P. Hubert, M. Fillet, Nonaqueous capillary electrophoresis method for the enantiomeric purity determination of S-timolol using heptakis(2,3-di-O-methyl-6-O-sulfo)-beta-cyclodextrin: validation using the accuracy profile strategy and estimation of uncertainty, *J. Chromatogr. A* 1120 (2006) 102–111.
- [20] A. Chaintreau, E. Cicchetti, N. David, A. Earls, P. Gimeno, B. Grimaud, D. Joulain, N. Kupfermann, G. Kuropka, F. Saltron, C. Schippa, Collaborative validation of the quantification method for suspected allergens and test of an automated data treatment, *J. Chromatogr. A* 1218 (2011) 7869–7877.
- [21] A. Combes, S. El Abdellaoui, C. Sarazin, J. Vial, A. Mejean, O. Ploux, V. Pichon, Validation of the analytical procedure for the determination of the neurotoxin β -N-methylamino-L-alanine in complex environmental samples, *Anal. Chim. Acta* 771 (2013) 42–49.
- [22] V. Cuzuel, J. Dugay, J. Brunet, A. Rey, J. Vial, V. Pichon, P.-L. Carrette, Validation of a liquid chromatography tandem mass spectrometry method for targeted degradation compounds of ethanolamine used in CO₂ capture: application to real samples, *Oil Gas Sci. Technol.* 65 (2014) 821–832.
- [23] European Union, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, *Off. J. Eur. Communities* 2002/657/E, 2002.

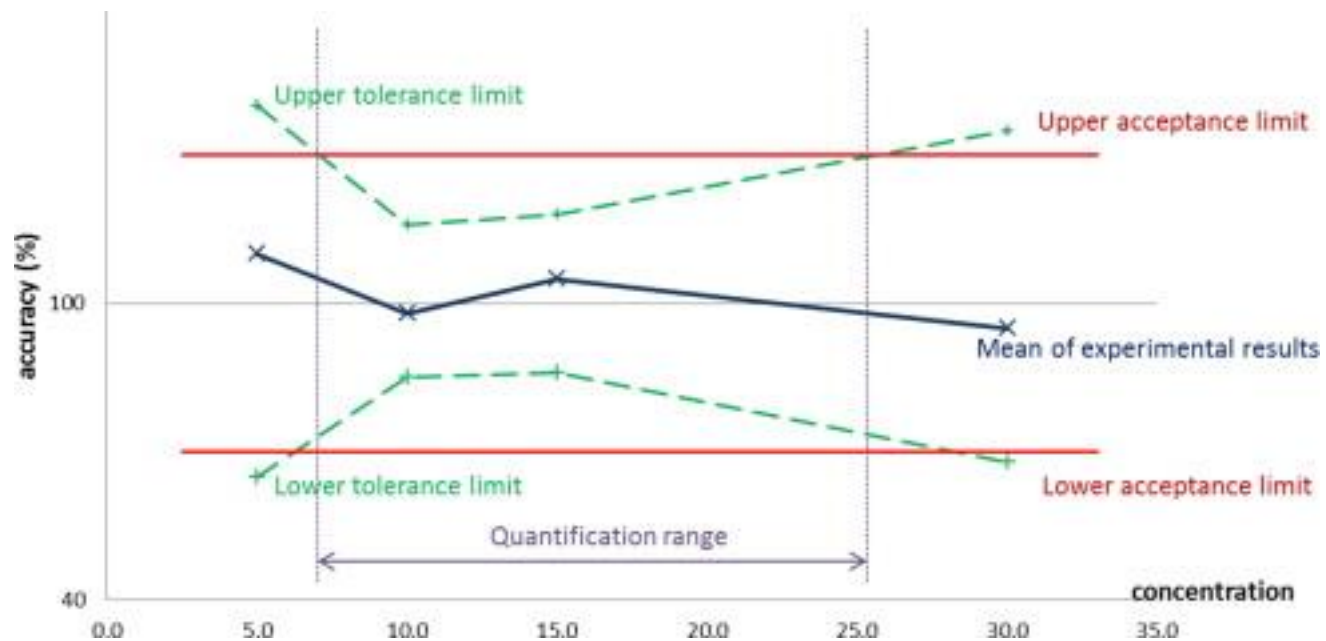


Fig. S1. Accuracy profile representation.

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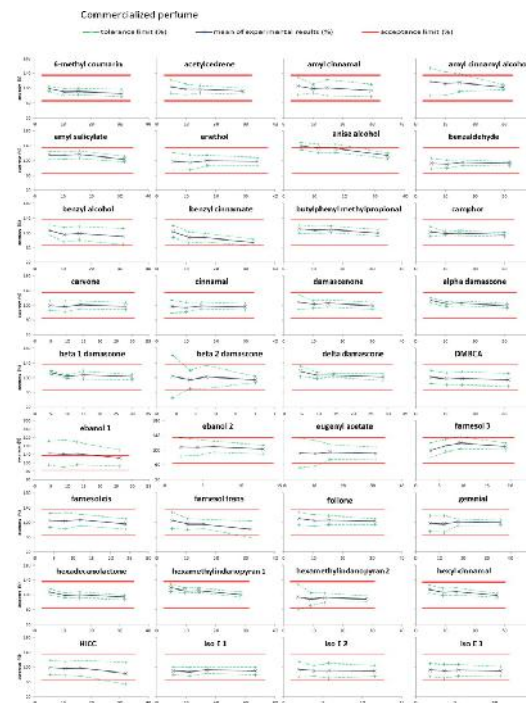


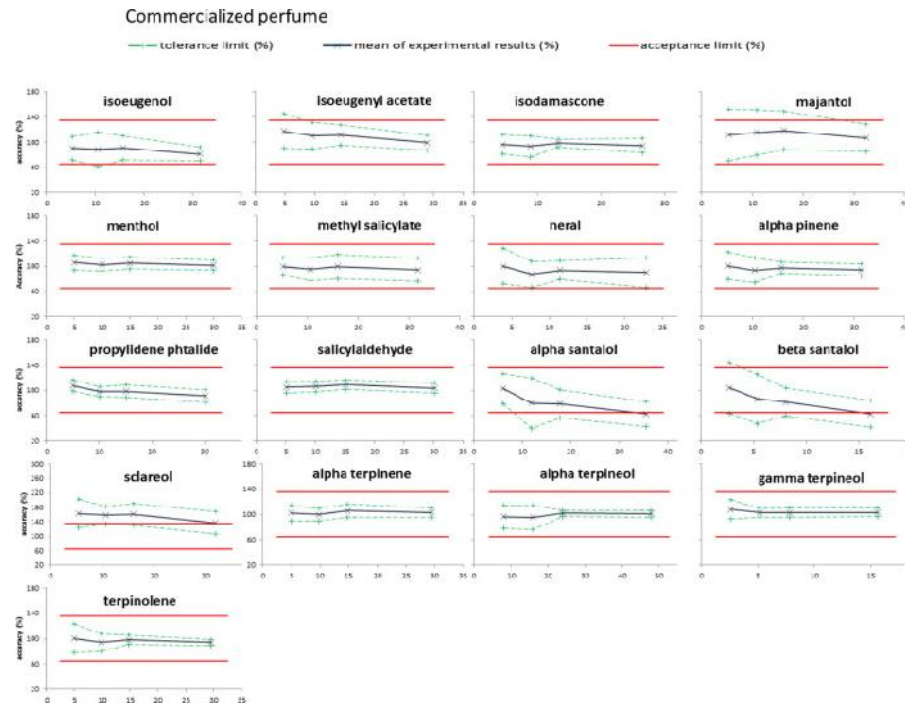
Fig. S2. Accuracy profiles of the 53 target analytes in matrix B.

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Table S1**Matrix A composition**

Raw material	Quantity (g)
ALDEHYDE C11 LENIQUE	0.4
ALDEHYDE C12 LAURIQUE	0.4
ALDEHYDE C12 MNA	0.4
ETHYL MALTOL	0.8
MALTOL	0.8
MELONAL	1.0
MANZANATE	1.2
ALLYLE AMYLE GLYCOLATE	2.0
BENZOATE METHYLE	2.0
OXANE	2.0
STEMONE	2.0
NEROLIONE	2.0
ORCINYL	2.0
ACETATE HEXYLE	2.4
ORANGER CRIST	2.4
ACETATE HEXENYLE CIS-3	2.6
ALDEHYDE CYCLAMEN EXTRA	3.0
ANTHRANILATE METHYLE	3.0
TRIPLAL	4.0
EVERNYL	4.0
AMBER CORE	4.6
ACETATE STYRALLYLE	6.0
CEDRAMBER	6.0
NORLIMBANOL	6.0
ACETATE ISOBORNYLE	6.0
VERDOX	7.0
MONTAVERDI	7.0
ACETATE PRENYLE	10.0
PTBCHA	10.0
KEPHALIS	10.0
AMBRETTOLIDE	10.0
HELIOTROPINE	12.0
DARTANOL	20.0
METHYLDIANTILIS	20.0
MYRRHONE NEO	20.0
CEDROL LIQUIDE	20.0
OPERANIDE	20.0
CASHMERAN	22.0
POLYSANTOL	22.0
SCLAREOLATE	24.0
ACETYLACETATE ETHYLE	25.0
JASMAL	30.0
LILYFLORE	32.0
SANDALORE	40.0
MUSCENONE	40.0
VETYVEROL	40.0
ETHYLENE BRASSYLATE	44.0
PEPPERWOOD	60.0
DIPROPYLENEGLYCOL	60.0
PAMPLEWOOD	68.0
DIHYDROMYRCENOL	260.0
Total	1000.0